Supplementary Information for

Dendrimer-encapsulated nanoparticle-core micelles as a modular strategy for particle-in-a-box-in-a-box nanostructures

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Experimental Section

Materials.

Amine-terminated polyamidoamino (PAMAM) dendrimers generation 7-9 were obtained from Dendritech Inc., MI, USA as 5 wt% methanolic solutions. $pMAA_{64}$-b-PEO_{885} (Mw/Mn = 1.15) was obtained from Polymer Sources Inc., Canada and used as a 5 mM solution based on carboxylic acid content. HAuCl4.3H2O was obtained from TCI. (3-(N-morpholino)-propanesulfonic acid) (MOPS), $NaBH_4$, 1M HCl and 1M NaOH solutions were obtained from Sigma Aldrich. NaOD, DCl and D2O with a purity of 99.96% D were obtained from Eurisotop, France.

Synthesis of Dendrimer Encapsulated Nanoparticles (DENs)

G7-Au_{256}DENs were made following established protocols.[10] Shortly, 50 µL (17 nmol) of 5 wt% PAMAM G7-NH_2 in methanol was transferred to a 5 mL vial and the solvent was evaporated under reduced pressure. Next, 2 mL of water was added to dissolve the PAMAM and the pH was adjusted to 3 using 1M HCl, after which 256 molar equivalents

of Au^{3+} to PAMAM were added as 1 mL of a 4.4 mM aqueous solution of $HAuCl_4$ at pH 3. The resulting solution was then stirred for 20 minutes, after which 44 µL of a 1M solution of $NaBH_4$ in 0.3M NaOH (10 molar equivalents to Au^{3+}) were added. This resulted in the reduction of Au^{3+} to AuDENs, indicated by the change from colorless to a dark brown solution within seconds after addition. After reduction, the pH was set to 7 using HCl and the Au_{256}DENS were stored at 4 ^{o}C as a 5.6 µM solution. To prove that the formed AuNPs reside on the inside of the PAMAM dendrimer (e.g. DENs as opposed to DSNPs) , 1H-NMR spectra were analyzed following the approach reported before.[11,12]

Dendrimer Encapsulated Nanoparticles in Micelles

To obtain dendrimicelles under charge stoichiometric conditions, 20 µL of G7-Au_{256} DENs (58 nmol positive charge based on surface groups) was dissolved in 149 µL water and 20 µL of 0.2M MOPS buffer at pH 7.0 was added. Then, 11 µL $pMAA_{64}$-b-PEO_{885} (55 nmol based on -COOH) was added and the sample was sonicated for two minutes. To obtain micelles at off-stoichiometric charge fractions, the amount of block copolymer and water added were adjusted accordingly, keeping the final volume constant at 200 µL. Samples were left to equilibrate for at least one day before characterization using cryoTEM. The nanoparticle-containing dendrimicelles used in this study are stable for at least months as concluded from high-resolution DLS and TEM analyses of fresh and aged solutions of dendrimicelles.

Characterization.

Dynamic Light Scattering (DLS) was done on a Malvern Zetasizer Nano S equipped with a laser operating at 633 nm. Sample grids for electron microscopy were obtained from Electron Microscopy Sciences (EMS, Hatfield, PA, USA) and were rendered hydrophilic using a plasma cleaning setup (15 s at 10^{-1} Torr). For cryoTEM, samples were cast on Quantifoil R2/2 grids or 400 mesh Holey Carbon grids. After blotting, samples were plunged into liquid ethane using a Vitrobot system (FEI Company). Samples were imaged at ~ 90K in a JEOL 2100 TEM operating at 200 kV. For normal TEM, solutions were deposited on hydrophilic 400 mesh carbon-coated copper grids. TEM image analyses was done using FIJI (https://fiji.sc/). Nuclear Magnetic Resonance (NMR) spectra in D2O were obtained at 300K on a Bruker 14.1 T Avance III spectrometer operating at 600.13 MHz for 1H, equipped with a 5 mm TXI cryoprobe.

Critical Micelle Concentration (CMC) calculation

The CMC was calculated from plotting the excess Rayleigh ratio (R_θ) as a function of the total polymer concentration, following the approach used by Wang et al.[16] The excess Rayleigh ratio is defined as: $R_\theta = \frac{\mathrm{I(sample) - I(solvent)}}{I(toluene)} * R(toluene) * \frac{n^2(solvent)}{n^2(toluene)}$, where I_{sample}, $I_{solvent}$ and $I_{toluene}$ are the scattered light intensity of the sample, the solvent and of a toluene reference, as obtained from DLS experiments. $R_{toluene}$ is the Rayleigh ratio of toluene and $n_{solvent}$ and $n_{toluene}$ are the refractive indices of the solvent (1.333) respectively the toluene reference (1.497).

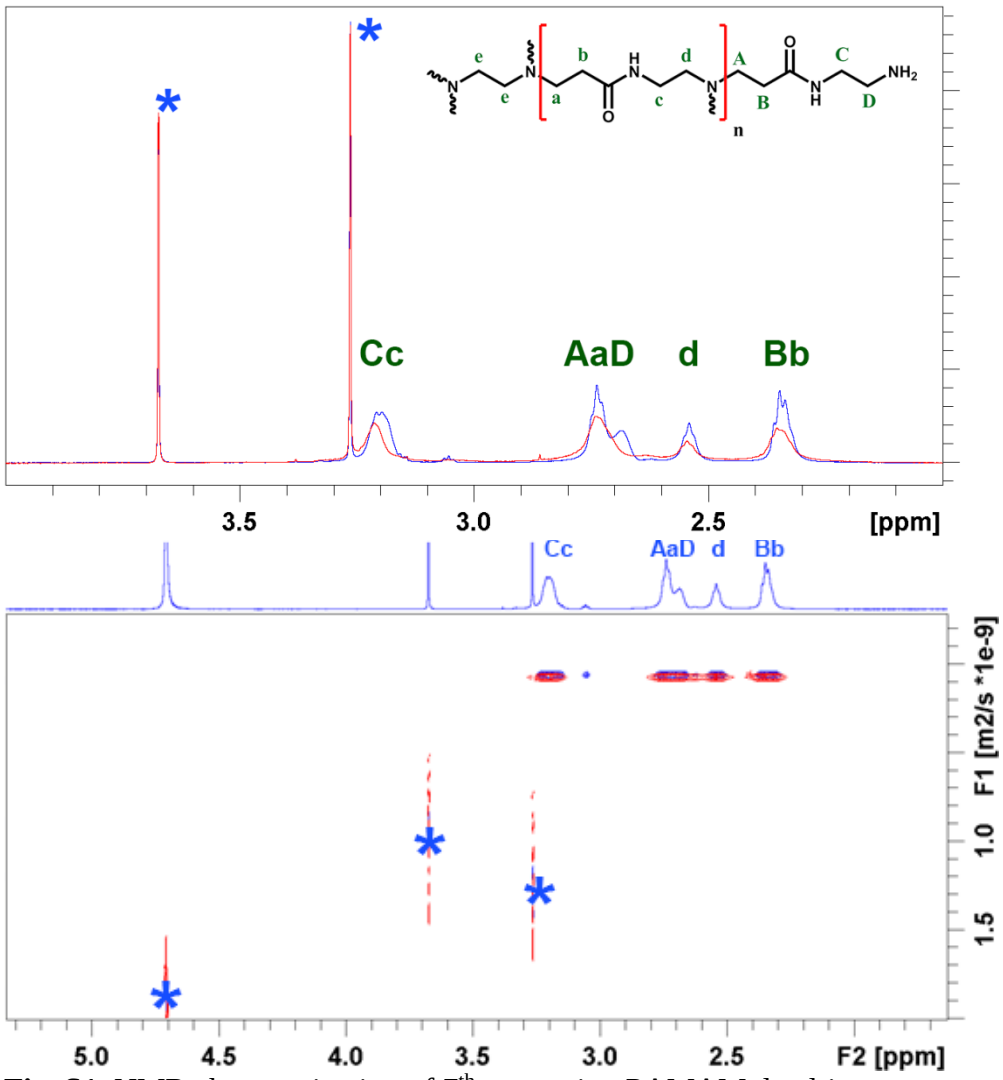

Fig. S1. NMR characterization of 7th generation PAMAM dendrimers.
Top: overlay of the 1H-NMR spectra of PAMAM-G7-NH2 (blue) and PAMAM-G7-Au_{256} (red) in D2O at pH 10. The increase of the ratio D/d from 1.0 to 1.2 for G7 to G7-Au_{256} proves that the Au_{256} nanoparticle resides inside the dendrimer cavity. The ratio D/d is calculated as the integral peak ratio of (AaD-Bb)/d.
Bottom: Diffusion-Ordered Spectroscopy (DOSY) spectra of PAMAM G7-NH_2 in blue and PAMAM G7-Au_{256} in red. The observed diffusion coefficient for G7 ($D = 4.29*10^{-11}$) corresponds to a hydrodynamic diameter of 9.8 nm. For G7-Au256, the observed diffusion coefficient ($D = 4.41*10^{-11}$) corresponds to a hydrodynamic diameter of 9.5 nm, indicating the absence of dendrimer stabilized nanoparticles. Peaks indicated with an asterisk belong to water (4.7 ppm), dioxane (internal standard, 3.7 ppm) and methanol (3.3 ppm). The pH of the samples was 10.

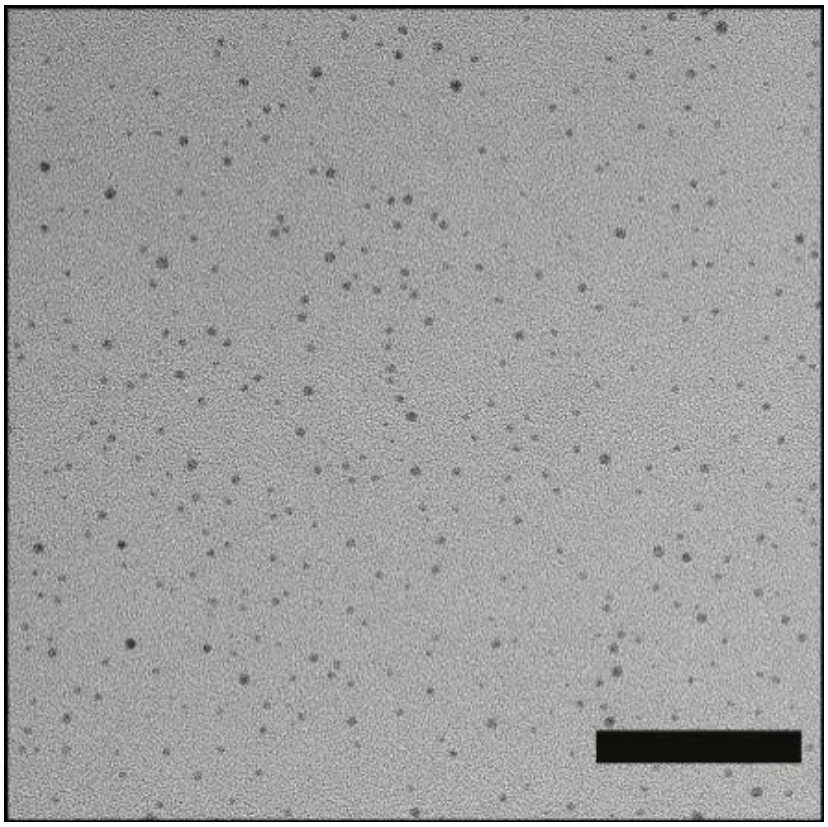

Fig. S2. TEM micrograph of PAMAM-G7 Dendrimer Encapsulated Nanoparticles (DENs). The G7-Au_{256} DENs are 1.8 ± 0.6 nm (226 particles analyzed). The scale bar in the figure corresponds to 50 nm.

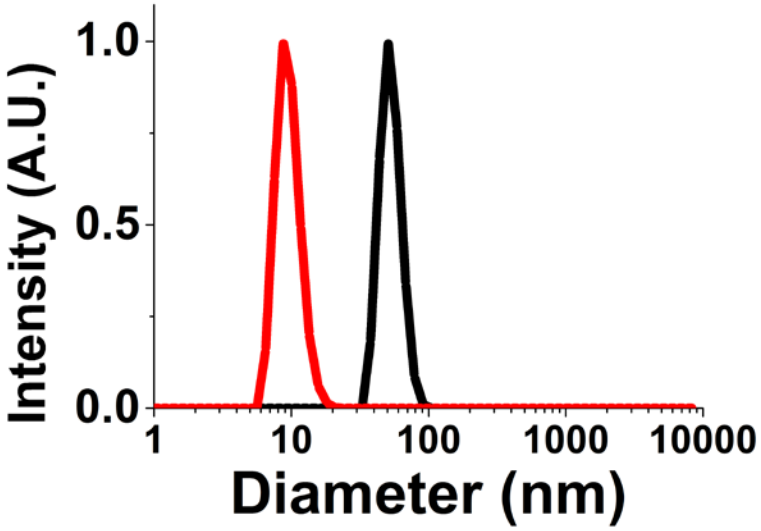

Fig. S3. DLS characterization of PAMAM G7 in solution and in dendrimicelles, showing the obtained number-averaged size plots. In red: the average hydrodynamic diameter of PAMAM in solution was about 10 nm. In black: the average hydrodynamic diameter of PAMAM dendrimicelles was about 50 nm.

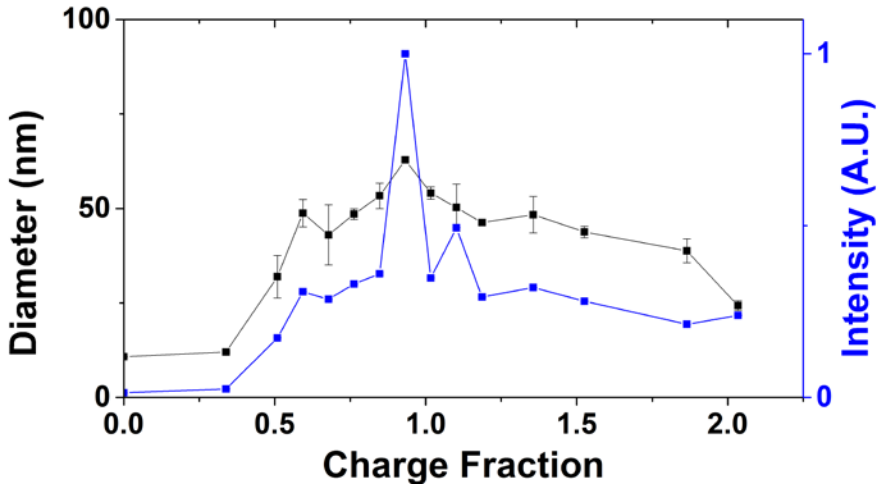

Fig. S4.
DLS charge titration graph of PAMAM G7-NH_2 with $pMAA_{64}PEO_{885}$, showing the number-averaged dendrimicelle diameter in black and the normalized scattered light intensity in blue against the charge fraction. For every data point, the amount of positive charge (dendrimer-NH2) was kept constant at 59 nmoles, and the amount of negative block polymer was varied, while keeping total volume constant. The charge fraction was calculated as the ratio of (NH2/COOH).

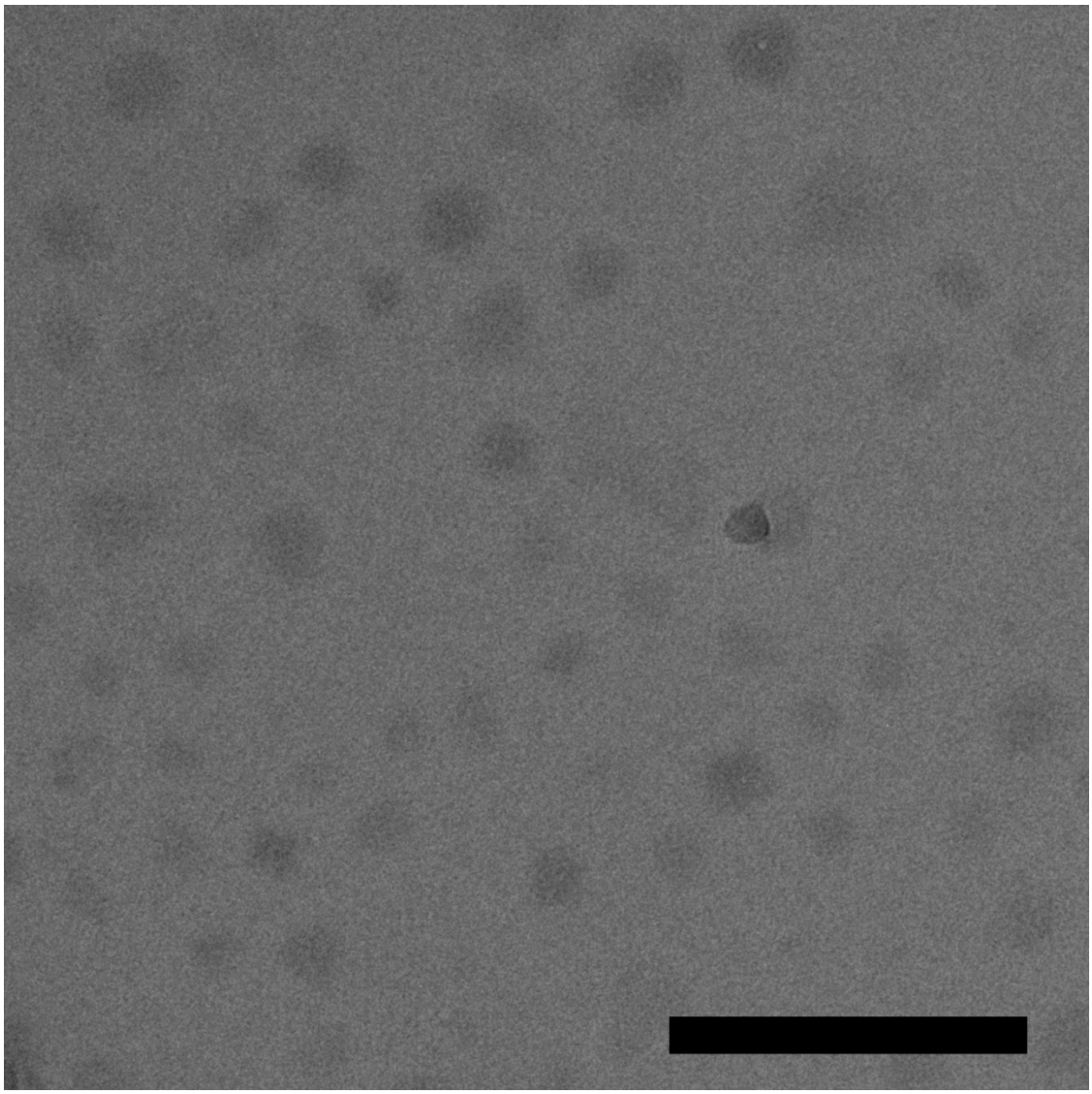

Fig. S5. CryoTEM micrograph of dendrimicelles made from empty, amine-terminated PAMAM G7. Scale bar is 200 nm.

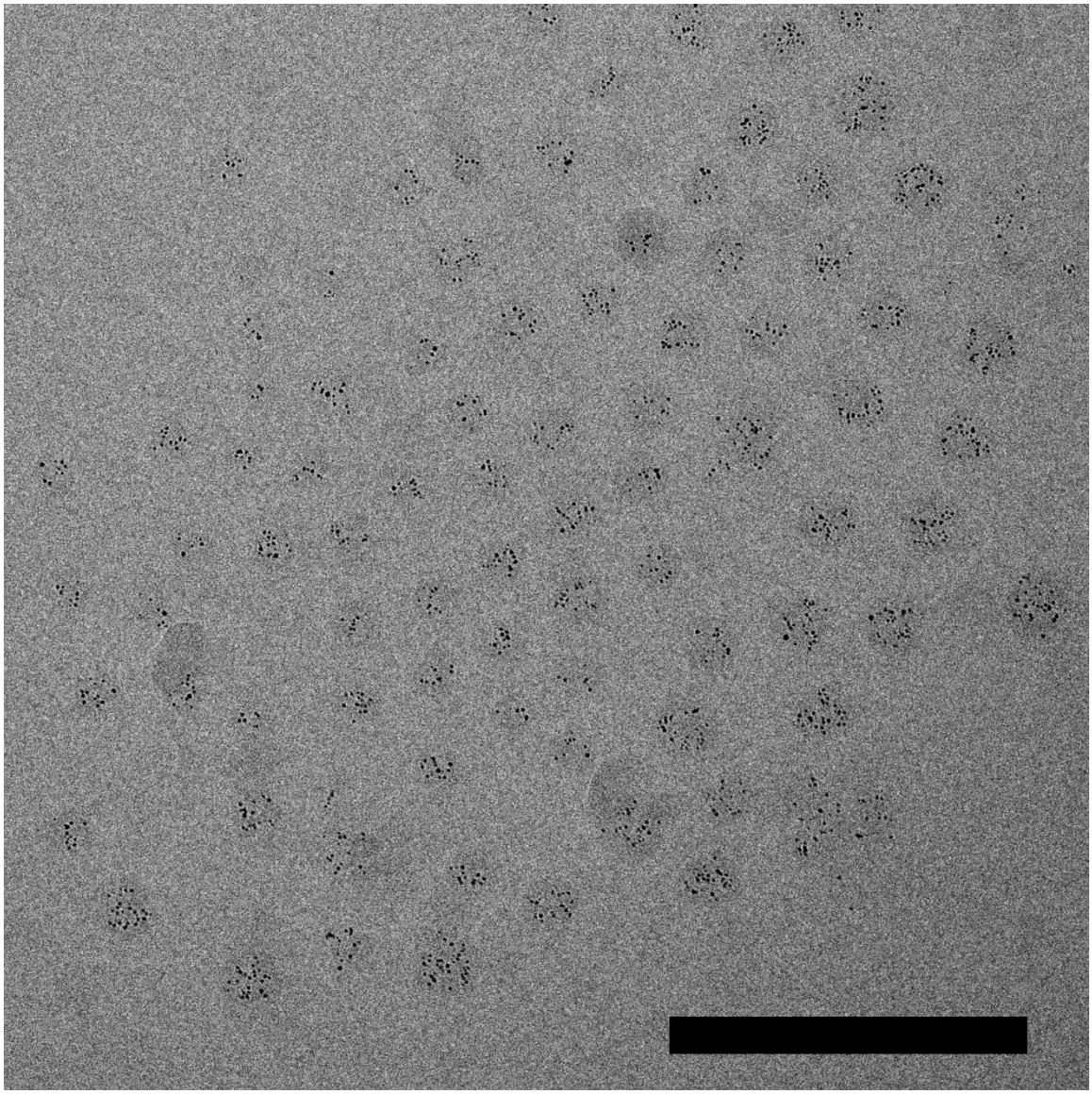

Fig. S6. CryoTEM micrograph of dendrimicelles made from 50% empty PAMAM G7 and 50% Au_{256}DENS. Scale bar is 200 nm.

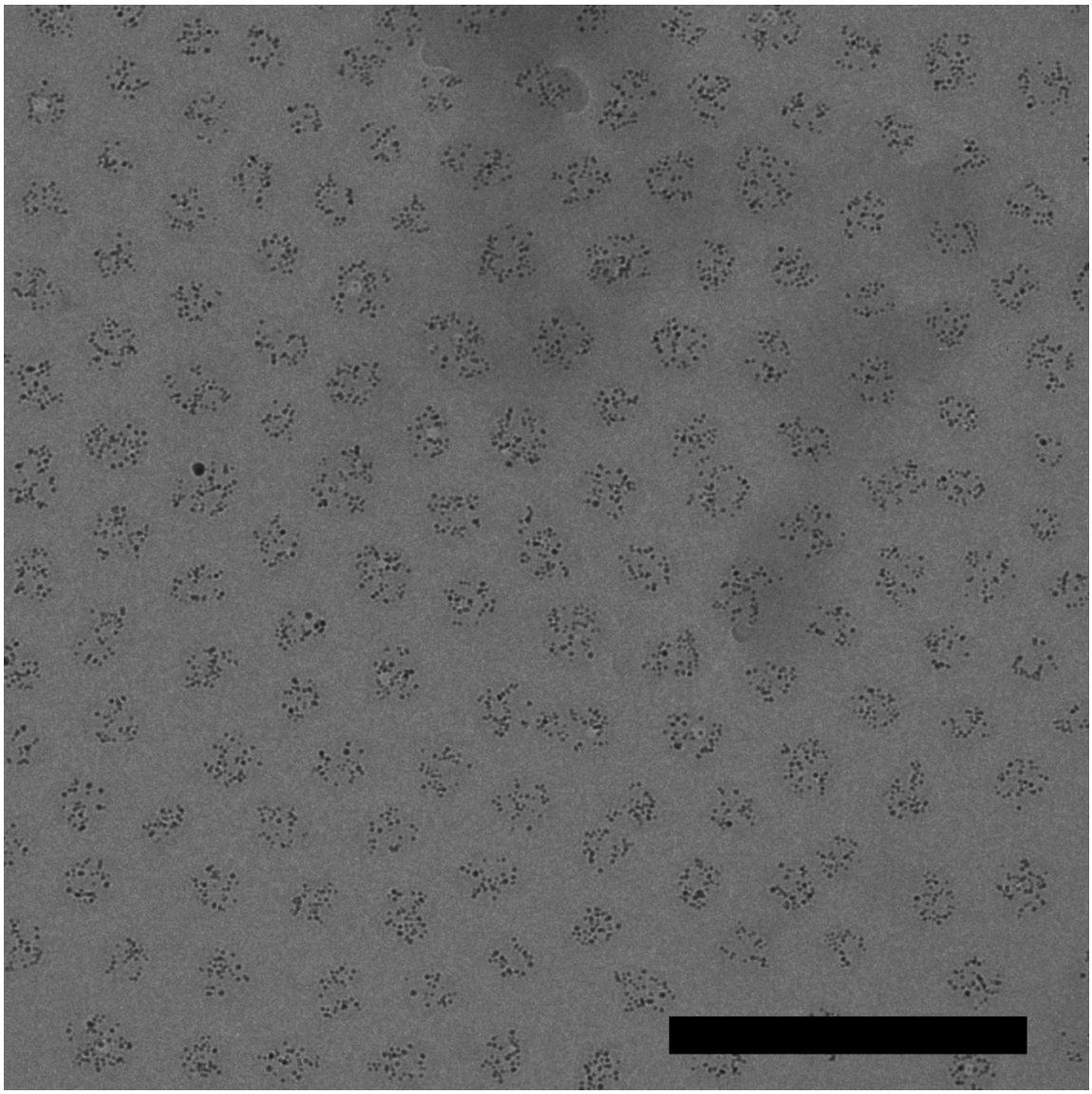

Fig. S7. CryoTEM micrograph of dendrimicelles made from Au_{256}DENS. Scale bar is 200 nm.

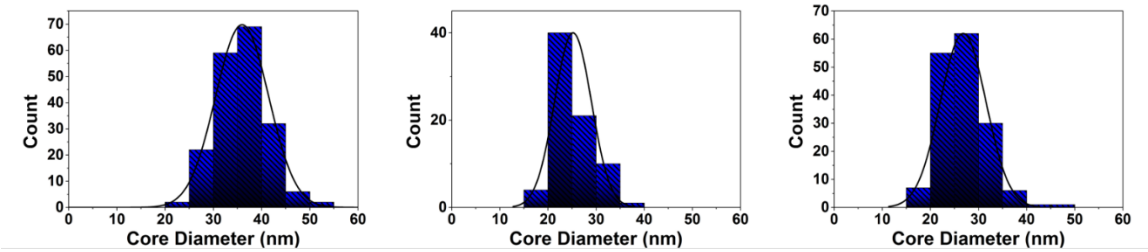

Fig. S8. CryoTEM characterization results of empty dendrimicelles, 50% empty / 50% Au_{256} dendrimicelles and Au_{256} dendrimicelles.
Left: Histogram showing the average micellar core diameter as obtained from the cryoTEM micrographs, for G7 dendrimicelles made under charge-stoichiometric mixing conditions. The average core diameter is 36 ± 6 nm (193 micelles analyzed). Middle: Histogram showing the average micellar core diameter as obtained from the cryoTEM micrographs, for 50% empty / 50% Au_{256} dendrimicelles made under charge-stoichiometric mixing conditions. The average core diameter is 25 ± 4 nm (76 micelles analyzed). Right: Histogram showing the average micellar core diameter as obtained from the cryoTEM micrographs, for Au_{256} dendrimicelles made under charge-stoichiometric mixing conditions. The average core diameter is 27 ± 5 nm (162 micelles analyzed).

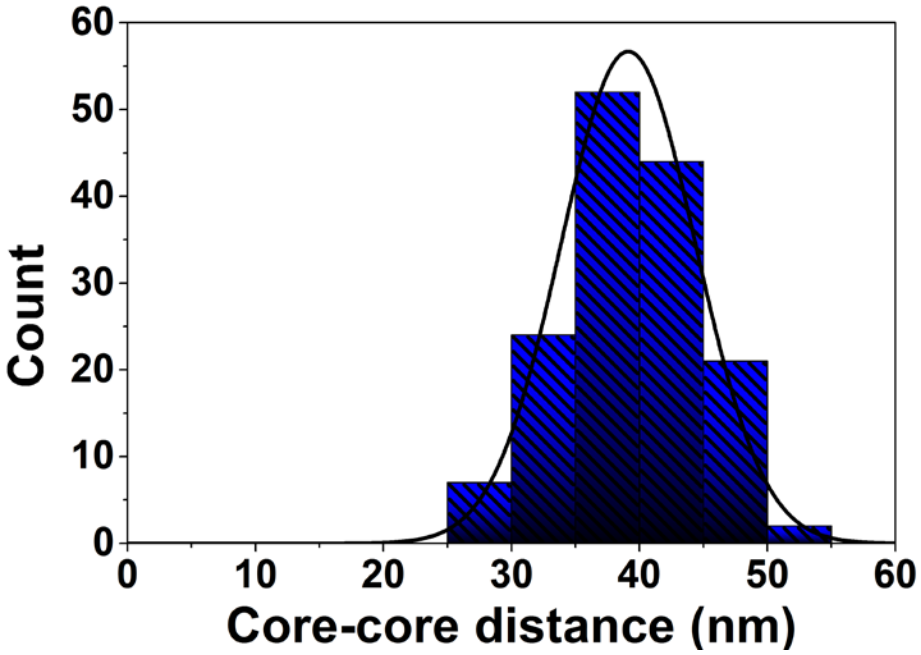

Fig. S9. Micellar size of G7-Au_{256} dendrimicelles as obtained from measuring the core-core distance of 150 randomly selected micelle pairs from the cryoTEM image in figure S6. The average core-core distance, and hence the micelle size (core+corona), is 39 ± 5 nm.

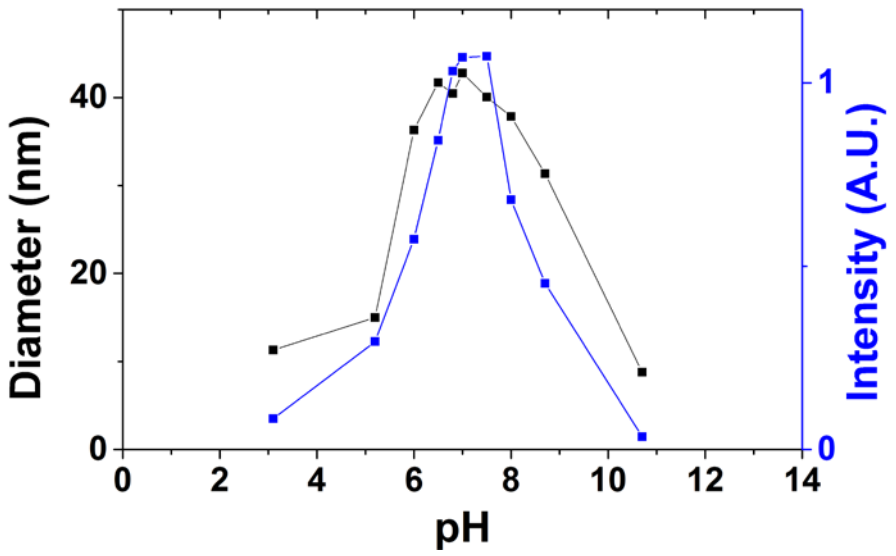

Fig. S10. The pH stability of G7 dendrimicelles. As can be seen, the formed dendrimicelles are stable between pH ~6 and ~8. In blue: Normalized average scattering intensity as obtained from DLS experiments. In black: number-averaged dendrimicelle size as obtained from DLS experiments. The dendrimicelles were made under charge-stoichiometric mixing conditions.

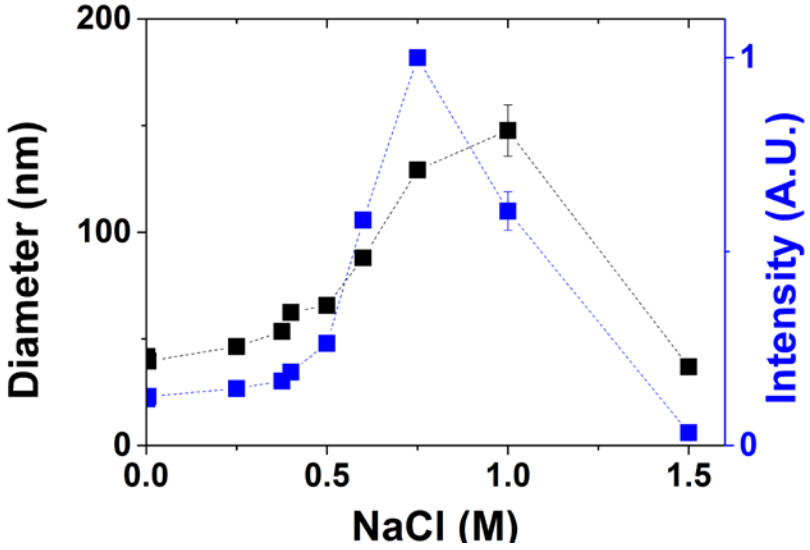

Fig. S11. Graph showing the stability of G7 dendrimicelles versus added NaCl. These dendrimicelles were made under charge-stoichiometric mixing conditions. In blue: Normalized average scattering intensity as obtained from DLS experiments. In black: number-averaged dendrimicelle size as obtained from DLS experiments. Up to ~0.5 M NaCl, both the scattered light intensity and the obtained size remain constant, indicating the stability of the dendrimicelles up to at least this salt concentration.

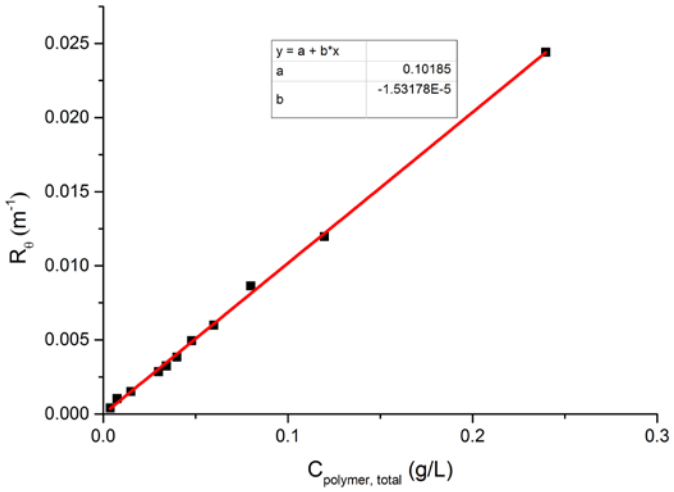

Fig. S12. CMC determination of G7 dendrimicelles. The excess Rayleigh ratio is plotted versus the total polymer concentration in gram per liter (e.g. the pMAApEO block copolymer concentration and dendrimer concentration). The data points were fitted linearly (see inset) and the CMC was obtained from the intersection with the x-axis.

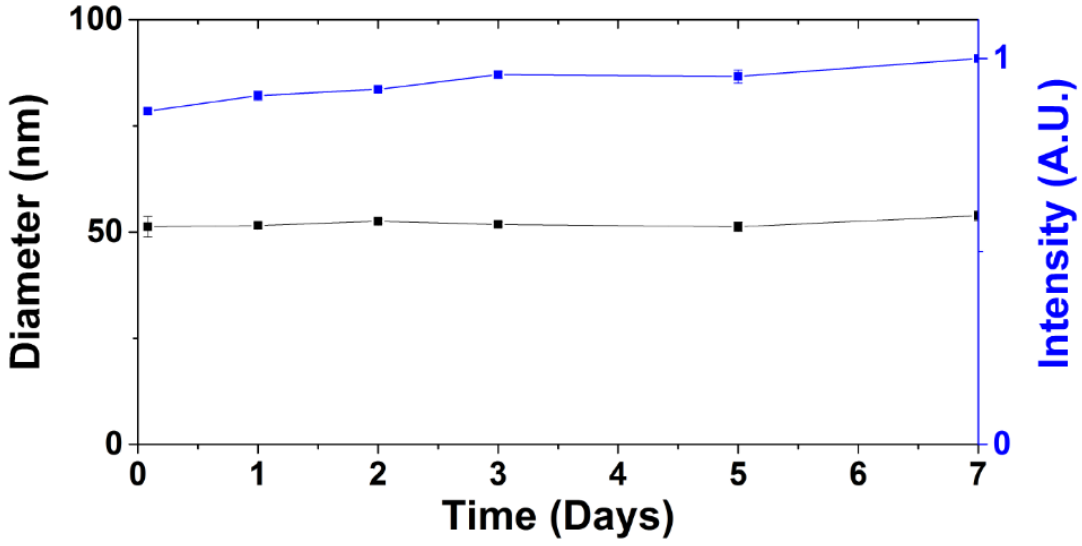

Fig. S13. The stability of G7-Au256 dendrimicelles over time. The average scattered light intensity and the average size remain virtually unchanged over time, indicating the stability of these dendrimicelles. In blue: Normalized average scattering intensity as obtained from DLS experiments. In black: number-averaged dendrimicelle size as obtained from DLS experiments. The dendrimicelles were made under charge-stoichiometric mixing conditions.

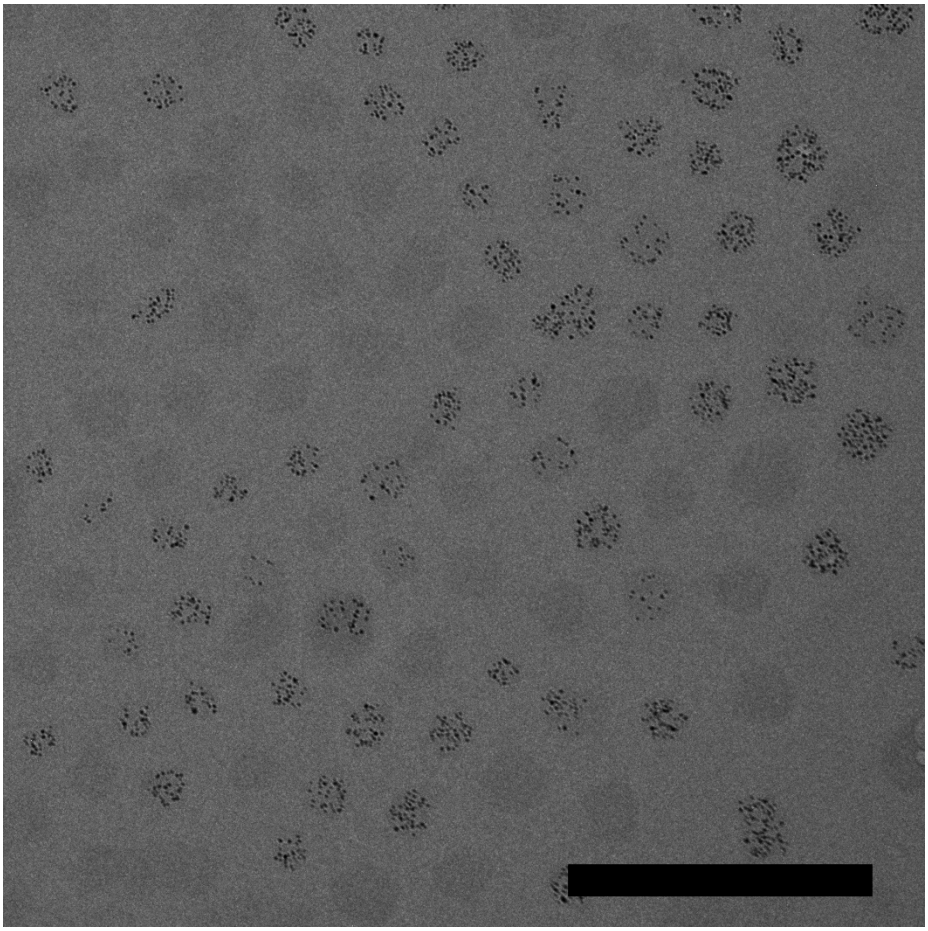

Fig. S14. Mixing empty and filled dendrimicelles does not result in half-filled micelles. This sample was made one day after mixing empty and filled dendrimers. As can be seen, the dendrimicelles did not exchange their core contents. The scale bar represents 200 nm.

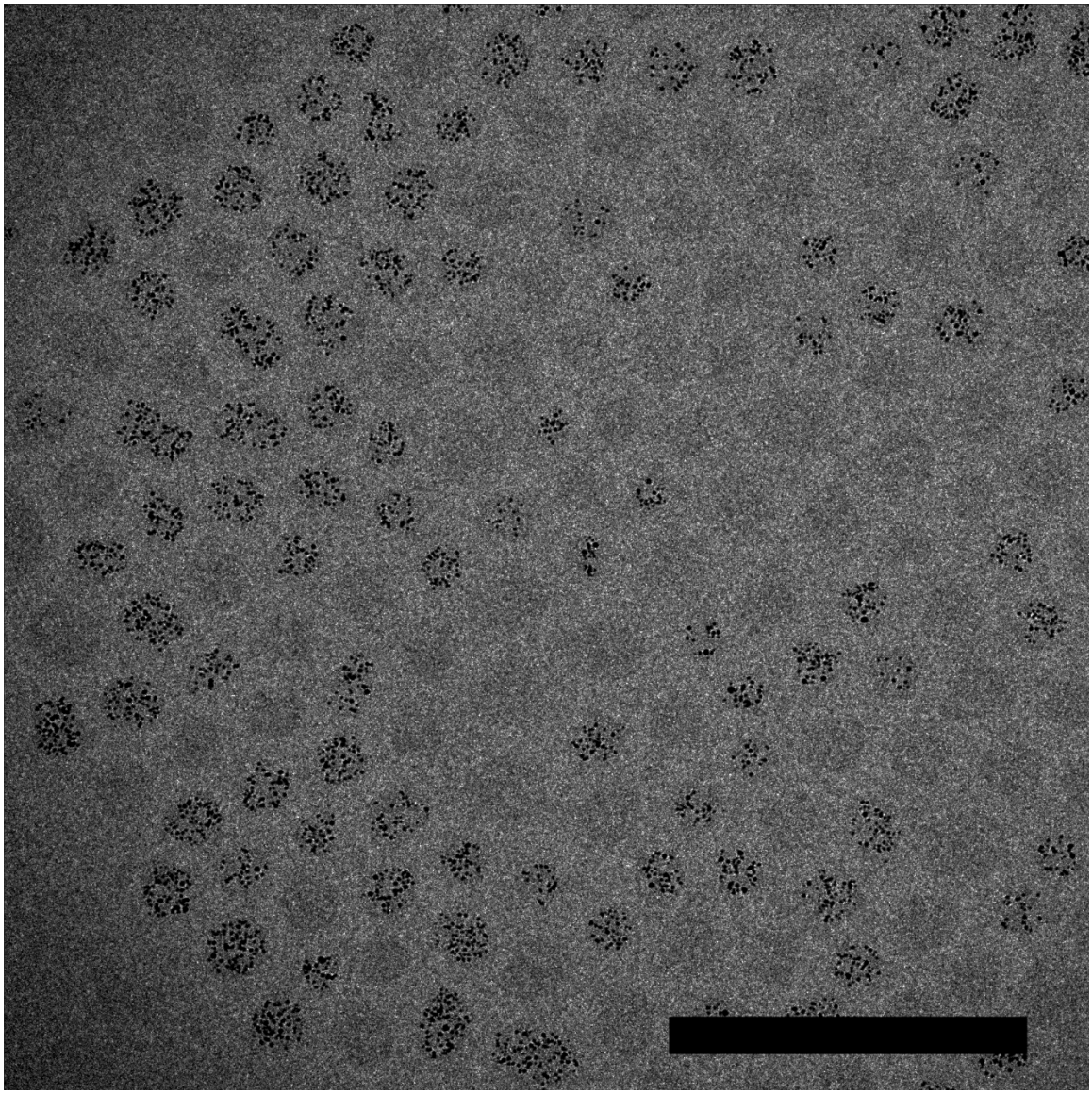

Fig. S15. Mixing empty and filled dendrimicelles does not result in half-filled micelles. This sample was made three months after mixing empty and filled dendrimers. The brightness and contrast in this image have been adjusted to better visualize the empty dendrimicelles. As can be seen, the dendrimicelles did not exchange their ‘core’ contents, even on extended timescales. The scale bar represents 200 nm.

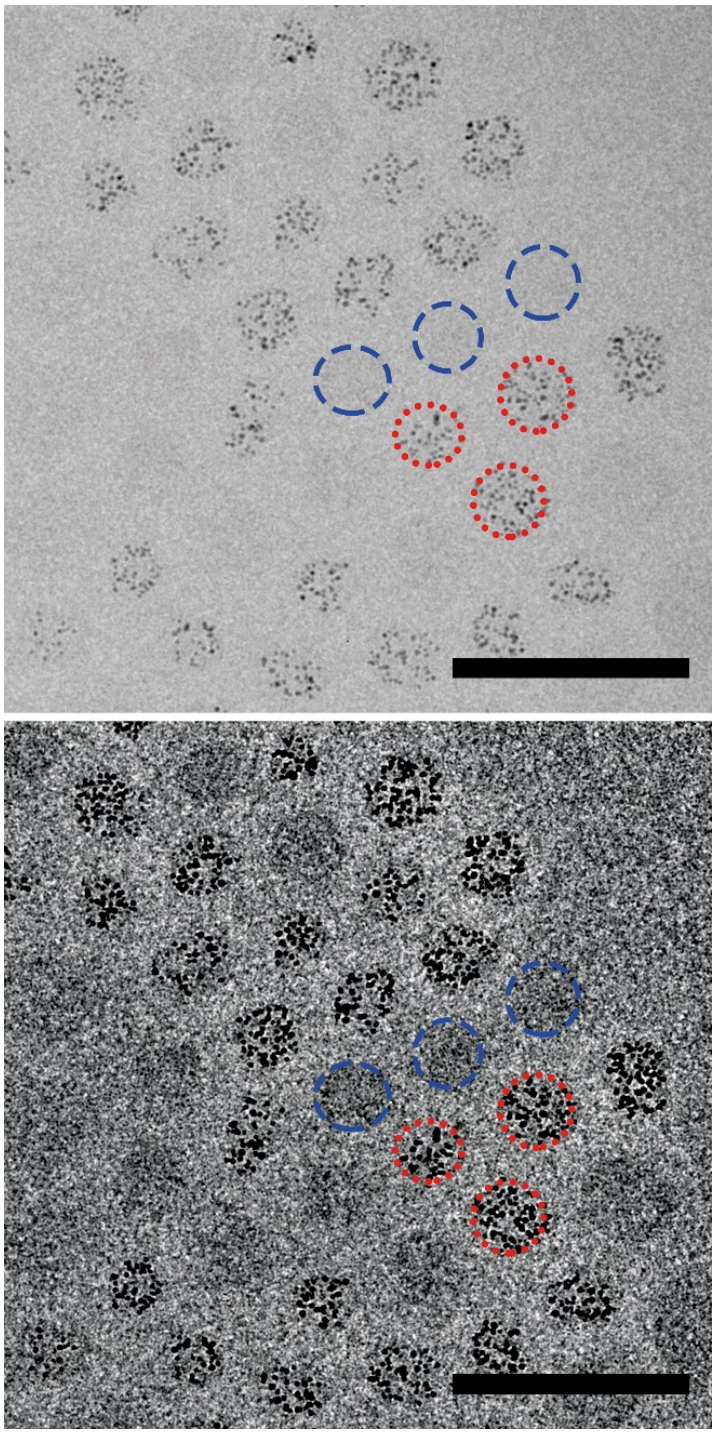

Fig. S16. Mixing empty and filled dendrimicelles does not result in half-filled micelles. Top: cryoTEM image with minimal post-processing. Bottom: The cryoTEM image has been corrected for uneven exposure by dividing the image by the Gaussian-blurred version (kernel size 15 nm) of the image, followed by linear adjustment of the brightness and contrast to such extent that the empty dendrimicelles are clearly visible as well. Scale bar in both images represents 100 nm.